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14. ABSTRACT
AXIMA Assurance mass spectrometer, Leica DMI-8 fluorescent microscope and BioRad V3 Western Workflow were purchased, installed and appropriate faculty and staff trained. Efforts were made to expand curricular offerings at CCP and provide opportunities for faculty and undergraduate student training and research including training sessions to make faculty aware of the care and use of the new equipment as well as the opportunities to incorporate the use of the V3 Western Workflow in their courses or research projects. The equipment was used in BIOL 241 (Microbiology) and will be used in BIOL 281 (Biochemistry). New Research opportunities have also been

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Report Title

Final Report: Molecular Analysis Research at Community College of Philadelphia

ABSTRACT

AXIMA Assurance mass spectrometer, Leica DMI-8 fluorescent microscope and BioRad V3 Western Workflow were purchased, installed and appropriate faculty and staff trained. Efforts were made to expand curricular offerings at CCP and provide opportunities for faculty and undergraduate student training and research including training sessions to make faculty aware of the care and use of the new equipment as well as the opportunities to incorporate the use of the V3 Western Workflow in their courses or research projects. The equipment was used in BIOL 241 (Microbiology) and will be used in BIOL 281 (Biochemistry). New Research opportunities have also been generated due to the availability of this research tool. Dr. Salerno entered into a collaborative research effort with The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine. He also recruited, trained and continues to perform a research project on innate immunity with a cohort of students titled: Themed inter-disciplinary training/ research experience: a pilot project to create co-curricular project-based learning opportunity for improving technical competence in STEM students at CCP. Students are training in techniques while concurrently engaging in a research project on the role of GADD45 in inflammation and septic shock using this equipment.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

Progress on the research projects presented below fall under the category of "molecular genetics", as presented in ARO Solicitation Number W911NF-12-R-0012-01. These projects expand current curricular offerings at CCP and also provide opportunities for faculty and undergraduate student research.

Aim1:

Every year, severe sepsis strikes approximately 750,000 Americans (Angus et al., 2001). It has been estimated that between 28 and 50 percent of these people die (Wood et al., 2004). This number, reaches approximately 200,000 deaths per year, and is far more than the number of U.S. deaths from prostate cancer, breast cancer and AIDS combined. Military personnel are frequently exposed to adverse conditions and injuries predisposing them to microbial infection and sepsis (Ingelsby et al., 2000).

The GADD45 family of genes is important in the regulation of myeloid cell differentiation (Reviewed in Hoffman and Liebermann, 2007). Therefore, it is essential to investigate role of GADD45 family of genes in the molecular mechanisms and cellular physiology involved in inflammation and sepsis. Recent evidence suggests that GADD45 family members play an important role in the inflammatory response of neutrophils and macrophages *in vitro* (Salerno et al., 2012). Novel approaches are needed to prevent acute sepsis in the clinic. Gadd45 proteins modulate p38, NF-Kb and JNK signaling (Figure 1), which play major roles in leukocyte activation (Salerno et al., 2012). Therefore, a better understanding of the role of GADD45 family members in macrophage and neutrophil activation should provide insight into the molecular mechanisms of inflammation and sepsis, leading to the development of novel therapeutics aimed at blunting the cytokine storm responsible for acute sepsis.

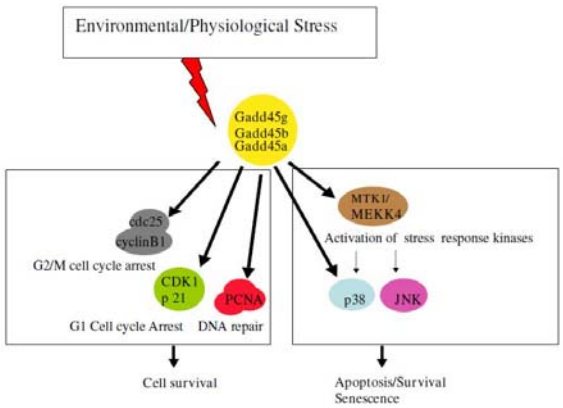

Figure 1. GADD45 family of genes plays important role in inflammatory stress kinase pathways.

Our recent studies have provided evidence that deficiency in either gadd45b or gadd45a impairs chemotaxis of BM (bone marrow) derived neutrophils and macrophages. Collectively, these findings justify further examination of the role GADD45 genes play in modulating the functions of the myeloid compartment in response to inflammatory stress. We hypothesize that each of the gadd45 proteins and their downstream effectors are required for the optimal innate immune response of myeloid cells, and that loss of any of the gadd45 functions will affect both the myeloid cellular and humoral inflammatory responses. Therefore, comparisons of WT vs. KO myeloid cell lines using bacterial surface proteins and cellular assays will allow us to further elucidate the role of the GADD45 family of genes in innate immunity and sepsis.

In addition to studying genetic components of the molecular response of myeloid cells to pathogens it is essential to examine changes in gene expression at the protein level to identify possible molecular targets for the development of therapeutics to downregulate inflammatory pathways in the goal of preventing sepsis. It has been established that the MAP kinase pathway (and others) plays an important role in macrophage and neutrophil activation. However, the

kinetics of these molecular signaling pathways in genetic variants (gene KO models) has yet to be elucidated.

To this end, we will employ various techniques to analyze the protein content of wt and GADD45 KO macrophage cell lines stimulated with various inflammatory stimuli in vitro. Firstly, Western Blot analyses will be performed on cell lysates to determine changed in the expression of known inflammatory pathway players like p38 MAP kinase, Nf-kB and JNK. The acquisition of multiple spectrophotometers for rapid quantification of cell lysate protein content will allow us to train students in their use more efficiently. Therefore we intend to purchase 6 spectrophotometers for student use.

V3 Western Workflow:

To better address the needs of our students and the time limits of the laboratory, we intend to use the V3 Western Workflow format (BIORAD) (Figure 2). The V3 Western Workflow format allows for rapid detection of specific proteins on the PVDF membranes using chemiluminescence. The V3 Western Workflow will enable us to perform Western blots within a 1-2 lab session time period, a significant improvement over existing technologies. This platform includes hardware and software for the collection of western blot images through chemiluminescence and quantification of protein bands. Analysis of images will also be performed using Adobe Photoshop software and image j.

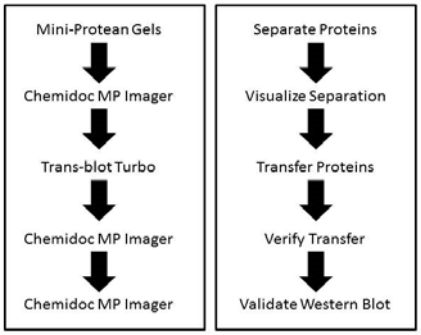

Figure 2. Workflow for BIORAD V3 Western Workflow Format. Equipment in left column, procedure in right column.

Progress: To this end, Community College of Philadelphia has completed the renovations and provided a specialized Research Laboratory in the West Building to house the V3 Western Workflow. The purchasing of the equipment was aligned with the completion of the Research Lab to insure the safety and proper installation of the equipment.

Additional progress has been made in the installation, training and incorporation of the V3 Western Workflow into our programs. Efforts have been made to expand current curricular offerings at CCP and provide opportunities for faculty and undergraduate student training and research. Subsequent to the procurement of the V3 Western Workflow, Dominic M. Salerno, Ph.D. was extensively trained by BioRad personnel in the care and use of the equipment. Dr. Salerno then set up multiple opportunities for training sessions to make faculty aware of the care and use of the new equipment as well as the opportunities to incorporate the use of the V3 Western Workflow in their courses or research projects. Six professors and two lab staff were trained on the V3 Western Workflow to ensure proper care and use of the equipment.

New Course documents have also been written and designed to incorporate the use of the V3 Western Workflow. Dr. Stuart Avart and Dr. Dominic Salerno designed a new course (BIOL) Biology 281 Biochemistry I which will use the V3 Western Workflow in

demonstrating differences in protein expression levels to Biology students. This course is being offered for the first time in the Fall 2015 semester.

New Research opportunities have also been generated due to the availability of this research tool. Dr. Salerno has entered into a collaborative research effort with Dr. Barbara Hoffman of The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, where their cutting edge resources and this new equipment may lead to exciting new findings in the field of innate immunity. Dr. Salerno has been granted full access to the research facilities at The Fels Institute starting in June of 2016. This will allow him to process sensitive mouse tissues, animals, prepare eukaryotic tissue culture samples and other approaches only available at R1 institutions. The project aims to validate the work of current graduate students and continue on Dr. Salerno's postdoctoral project (Salerno et al., 2012, Salerno et al., unpublished). The continuation of this project aims to understand the role of GADD45 family members on the innate immune response *in vivo.* It aims to do so through using mouse models of inflammation and examining the effects of LPS (Lipopolysaccharide) on myeloid cells ex vivo through inflammatory cytokine arrays.

In addition, Dr. Salerno has recruited, trained and continues to perform a research project on innate immunity with a cohort of students. The project is titled: Themed inter-disciplinary training/ research experience (TITRE): a pilot project to create co-curricular project-based learning opportunity for improving technical competence in STEM students at CCP (See Training Component below). The goals of this project are as follows: (i) To enhance STEM student experience and retention by creating opportunities for themed interdisciplinary training/ research experiences (TITRE). (ii) to run a pilot project around the theme "inflammation" which along with other projects, will form the basis of seeking external funding for approaches that would improve students learning and success in STEM courses. The V3 Western Workflow equipment serves as an essential tool in completing this research project. Funds for training students on the V3 Western Workflow were obtained through a Community College of Philadelphia Foundation Grant. A more complete description of the proposed research can be found at the end of the report.

Phase Contrast Fluorescence Inverted Microscope with 5MP CCD Fluorescence Camera

Fluorescent microscopy is a powerful tool in demonstrating the immunologic capacity of myeloid cells to students, who are able to see immune activation occurring in fixed/stained as well as live specimens. The Inverted Laboratory Microscope with LED Illumination from Leica is a cost-effective, versatile and powerful tool capable of providing students the opportunity to gain practical experience in slide preparation, microscopy and imaging. This setup allows the students to observe live cell cultures (visible) and obtain images of fluorescently labeled proteins or fluorescently stained organelles and structures. Students will be trained to use the fluorescent microscope using fixed, stained specimens and by performing phagocytosis assays with paramecium and fluorescently stained yeast. In addition, phagocytosis assays will be employed to determine the capacity of macrophage and neutrophil cell lines to respond to gradients of inflammatory stimuli to confirm preliminary findings.

Progress: **To this end, Community College of Philadelphia completed the necessary renovations and provided a specialized Research Laboratory in the West Building to house this fluorescent microscope. Purchase orders for the Inverted Laboratory Microscope with LED Illumination from Leica were placed with the purchasing department of the Community College of Philadelphia. The purchasing of the equipment was aligned with the completion of the Research Lab to insure the safety and proper installation of the equipment.**

Subsequent to the purchase and installation of the Leica DMI-8 Fluorescent microscope, extensive progress has been made in training and utilization. Dr. Salerno was trained extensively by Leica personnel in care and use of this new equipment. Dr. Salerno provided opportunities for individualized training for faculty and students interested in using the new equipment. Dr. Salerno has scheduled a professional development seminar for Biology faculty for training in the care and use of this equipment as well as incorporating use of the equipment in their curriculum. Dr. Salerno is currently compiling of gallery to cell and tissue sample images as source material for publishing a photo atlas for anatomy and physiology (BIOL 109) and Microbiology (BIOL 241) courses.

Protocols were optimized for the fixation, staining and imaging of cells in culture as well as fixed specimens from mouse tissues (Salerno et al., unpublished data). Agreements have been made with Barbara Hoffmann (Temple University) for collaboration in attaining eukaryotic cell specimens and mouse tissue samples used by Dr. Salerno in work demonstrating the importance of GADD45 in the inflammatory response and septic shock in mice (Salerno et al., 2012).

Aim 2:

Schiffman (2012) recently presented detailed rationale for further research into the cellular and molecular actions of sucralose, a popular artificial sweetener which may have negative impacts on human health. Sucralose is marketed under the trade name Splenda. Sucralose is a synthetically chlorinated form of natural table sugar, sucrose (Fig. 3). Since the molecular structures of sucrose and sucralose are similar, sucralose may be mistaken for sucrose within the cell. Sucralose may therefore interfere with the normal actions and metabolism of sucrose, or other similar small carbohydrates, such as glucose, fructose, maltose, and others. Preliminary data (Wagner-Graham, 2013, unpublished) supports the theory that sucralose may compete with sucrose on a molecular level, as sucralose has been observed to inhibit invertase, an enzyme which breaks down sucrose in eukaryotic cells.

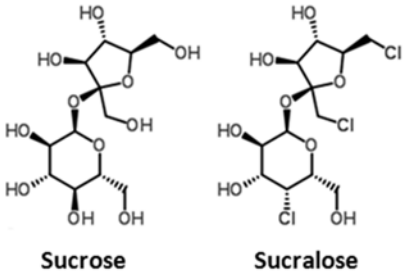

Figure 3. Sucralose and sucrose have similar chemical structures. Sucralose contains chlorine atoms at three positions, in place of the oxygen atoms found in sucrose. (Chemspider 2013)

The degradation of sucralose by environmental bacteria has been demonstrated (Labare and Alexander 1994). Perhaps bacteria in the human intestinal flora may also degrade sucralose. The predicted breakdown-products of sucralose contain aldehyde groups (Labare and Alexander 1994). Unsaturated aldehydes are reactive and capable of chemically modifying both DNA and proteins.

The chemical modification of proteins present in tissues lining the intestinal tract could lead to loss of normal function, which may result in disease. Sucralose alone has been implicated in fostering inflammation in irritable bowel syndrome (IBS) in humans (Qin, 2011). Sucralose alters normal intestinal flora in rats, notably reducing the numbers of anaerobic bacteria (Abu-Donia *et al*. 2008). This finding suggests sucralose exerts antimicrobial properties, but the molecular action is unknown. More information regarding the molecular antimicrobial action of sucralose may lead to development of novel antimicrobial drugs.

Comparative 2D gel electrophoresis (Figure 4) is a versatile technique that can be applied to multiple different cell types and systems. This approach can reveal changes in the proteins produced by cells in response to exposure to sucralose (or its break-down products). The changes in protein production are reflective of changes in gene regulation. The data indicate how protein production changes in cells exposed to sucralose, which may indicate perturbation of carbohydrate metabolism in those cells. Further, any chemical modification of proteins by sucralose (or its break-down products) would be evidenced as altered protein spot location on 2D gels. Before performing open-ended research into the effects of sucralose on cells, students would validate their technical execution of 2D gel electrophoresis using *Escherichia coli* cells, by comparing their results to those contained in the EcoProDB Database (Yun *et al*. 2007), which contains model 2D gel images for *E. coli*.

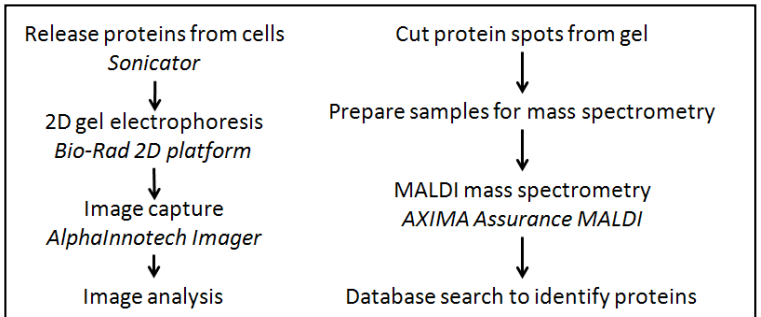

Figure 4. Workflow for 2D gel electrophoresis and peptide mass fingerprinting. The use of requested equipment is italicized.

Identification of proteins by peptide mass fingerprinting is accomplished using techniques which mirror those used previously by MA Wagner-Graham in successful proteomic analysis of *Brucella* spp. (Wagner *et al*. 2002) and *Bacillus anthracis* (Lamonica *et al*. 2005). After image analysis, protein spots of interest are excised from the gel and processed for peptide mass fingerprinting by digestion with trypsin and chemical modification with O-methylisourea. Peptides are then mixed with a matrix such as α-hydroxycinnamic acid, and spotted onto a metal target plate for analysis by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. Students will validate their ability to successfully prepare proteins for peptide mass fingerprinting and perform MALDI mass spectrometry by peptide mass analysis of cytrochorme c, as described by Beardsley and Reilly, 2002.

Two-dimensional (2D) gel electrophoresis platform and MALDI mass spectrometer

A two-dimensional (2D) gel electrophoresis platform and peptide mass fingerprinting using a matrix-assisted laser desorption ionization (MALDI) mass spectrometer would provide novel instructional and research capabilities to the faculty and students at CCP. The proposed 2D gel electrophoresis platform is manufactured by Bio-Rad.

The AXIMA Assurance MALDI mass spectrometer manufactured by Kratos Analytical would be used to identify proteins of interest by peptide mass fingerprinting. Masses of the peptides are measured using the mass spectrometer. To identify proteins, individual peptide mass fingerprints are matched against a theoretical database containing all possible peptide mass fingerprints produced by the cell type employed in the study.

The acquisition of this equipment will greatly enhance the basic science instruction and research capabilities of our existing lab facilities. In combination with existing tools, a sophisticated training and research experience will be provided to students that will prepare them for careers in fields ranging from basic science research to medicine. The acquisition of the requested equipment would extend our capacity to instruct students in protein biochemistry, cell and molecular biology and many other disciplines.

Progress: **The Community College of Philadelphia completed the necessary renovations to provide a specialized Research Laboratory in the West Building to house the Axima Assurance. The AXIMA Assurance MALDI mass spectrometer manufactured by Kratos Analytical (Shimadzu) was purchased and installed. The purchasing of the equipment was aligned with the completion of the Research Lab to insure the safety and proper installation of the equipment.**

Dr. Mary Ann Wagner-Graham and Dr. Salerno were trained in the use and care of the AXIMA Assurance MALDI mass spectrometer. Training sessions were offered to interested faculty. Dr. Wagner-Graham was able to use the mass spectrometer in her microbiology course (BIOL 241). Students used the MALDI to demonstrate that the MALDI mass fingerprints for bacteria were changed in response to different artificial sweeteners (Sucralose). Dr. Wagner-Graham, Dr. Powell and Dr. Celenza are currently working on a proposal with Philadelphia University to serve as a basis for an institutional partnership in proteomics research between The Community College of Philadelphia and Philadelphia University.

Training Component:

4-5 students were chosen for the research experience through competitive application. Students were chosen from 2nd year STEM major students and post-baccalaureate students considering careers in the life sciences. Ideally, the students will experience an immersive lecture and training period of two weeks followed by 8 weeks of additional training and laboratory exercises. The initial two week training period will provide students with a theoretical understanding of inflammation, cell signaling, protein isolation techniques and a survey of the existing literature on GADD45 and inflammation. Students will be required to perform literature searches in conjunction with the learning center of The Community College of Philadelphia and make summaries and criticisms of the journal articles chosen for review.

The project aims to elucidate the gene expression pathways and patterns responsible for myeloid innate immune responses to pathogens involved in sepsis. Additionally, this project will address the molecular pathways essential to resolution of inflammatory responses in vitro.

Students are in the process of validating and expanding upon previously published results (Salerno et al, 2012). Students were given specific tasks in the laboratory, following well-established protocols provided by the instructor. Students are expected to learn and apply techniques for SDS-PAGE gel electrophoresis and gene products which are up- or down-

regulated in response to inflammatory stimuli. To this end, students made solutions, lysed cells, quantified protein of cell lysates, prepared lysates for gel electrophoresis, and performed Western blotting techniques. Students are also expected to master the use of fluorescent microscopes in the study of macrophages and neutrophils stimulated with inflammatory molecules such as LPS (lipopolysaccharide). The work will train students to understand techniques used in investigating the signaling pathways that are involved in innate immunity and inflammation.

Students will be assessed through several approaches. Upon completion of the initial two week training period, retention of the theoretical training will be assessed using a standard exam with an emphasis on protein biochemistry and practical skills. During the lab intensive portion of the summer research program, students will be assessed on their ability to perform various laboratory techniques in an efficient and productive manner using a standard rubric. A final assessment of the student's work will be completed at the end of the training program to determine the quality of the overall body of work. Students will complete SALG-based on-line questionnaires to track learning gains.

Students who complete the program are expected to keep diligent and detailed notes on the research performed in the laboratory. Students are expected to compose comprehensive research report at the conclusion of the 10 week period. Students are expected to present their findings in a poster session in combination with an oral presentation at an existing venue at the college, at the discretion of the department chair. Students will present their findings at the Science Week at The Community College of Philadelphia or during regional and national conferences. Students who meet the minimum requirements above will be given departmental distinction, a high honor in our program.

In addition, the research program will attempt to reach out to other faculty to train them in the techniques for SDS-PAGE gel electrophoresis, microscopy and 2D gel electrophoresis, in hopes that they would incorporate these techniques in their lab activities. Faculty will be trained in 2 week laboratory sessions which can then be incorporated into their existing curriculum. This will allow us to reach many more students through existing courses offered by Biology Department of The Community College of Philadelphia. The expected outcome is that faculty will become familiar with the theory and techniques employed in cell signaling, and are able to bring that information to their students. Faculty completing the seminar(s) will then have the option to incorporate protein-based or microscopy lab activities into their existing courses. At this time, no high school outreach program is planned for this equipment. Long term goals for the Department of Biology include outreach to the Philadelphia School District to improve the quality of STEM education for underprivileged groups.

<u>Progress:</u> Although this award did not offer support for the training of faculty, staff or students, Dr. Salerno obtained a Foundation Grant from the Community College of Philadelphia in support of such training. The purpose of this grant is to enhance retention and success of science, technology, engineering and math (STEM) students at Community College of Philadelphia (CCP) through a mentored research experience. Based on the assessment of learning gains during this experience, it is our goal to seek additional external grant funding for approaches identified that would improve students' learning and success in STEM courses. After training of involved faculty in the use of the equipment funded in this grant was completed, the research equipment supported a pilot study to investigate the scientific goals in Aims 1 and 2.

The Foundation Grant described above is currently being implemented at The Community College of Philadelphia. The timeline for completing the grant is as follows:

Grant Period (Start and End Dates): August 2014 – July 2016

Project Timeline

Semester 0 Fall 2014

Project activity

1. Preparations: Purchase reagents, set up standards, develop protocols for safety and procedures for students.
2. Test run equipment and preliminary samples
3. Recruitment: Advertise the TITRE project, recruit and screen and select 6 students (split into 2 cohorts, A and B of 3 students each)

Semester 1 Spring 2015

Project activity

1. Pre-survey or students preflection [1]
2. Training.
    A. Scientific method review
    B. Techniques: Western Blot (NERC)

Semester 2 Fall 2015 (or summer)

Project activity

1. Training (techniques). PCR … (NERC/MAIN)
2. Mentored team project continued
3. Assess students competency using modified OSATS questionnaire

Semester 3 Spring 2016

Project activity

1. Post-survey of students experience
2. Dissemination

- Student presentation Science week
- Faculty presentation Spring 2016 Professional Development week

3. Summarize findings and submit reports

Presently, we are training students in techniques while concurrently engaging in a research project on the role of GADD45 in inflammation and septic shock. The proposed date of completing the mentored research experience is the Spring of 2016. As reported above, the first two portions of the foundation grant have been developed and completed. All needed equipment has been purchased and training of faculty has been completed. All students have been recruited and trained. The research project will continue over the summer and into the Fall 2015 semester. Upon completion of the lab research and analysis of data, a poster presentation and seminar will be created to communicate scientific findings at The 2016 American Society for Microbiology's annual meeting in the Spring 2016 semester. Findings will also be presented during Science week and as part of Spring 2016 Professional Development. Funds to support this extended time were included in the budget of The "Themed Interdisciplinary Training/Research Experience" Foundation grant (# 761056-F40000).